

CLAIMS

What is claimed is:

1. An isolated and purified platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide.
- 5 2. The isolated and purified platelet VDCC α_1 subunit polypeptide of claim 1, wherein the polypeptide comprises:
 - (a) a polypeptide encoded by a nucleic acid molecule having the nucleotide sequence set forth as any of SEQ ID NOs:1 , 3, 5-8, 28, or 29;
 - (b) a polypeptide encoded by a nucleic acid molecule that is substantially 10 identical to any of NOs:1 , 3, 5-8, 28, or 29;
 - (c) a polypeptide having the amino acid sequence set forth as SEQ ID NO:2 or 4;
 - (d) a polypeptide that is a biological equivalent of the polypeptide of SEQ ID NO:2 or 4; or
 - 15 (e) a polypeptide which is immunologically cross-reactive with an antibody that shows specific binding with a polypeptide of SEQ ID NO:2 or 4.
3. The polypeptide of claim 1, wherein the platelet VDCC α_1 subunit polypeptide comprises a platelet VDCC α_1 S subunit polypeptide or a platelet VDCC 20 α_1 D subunit polypeptide.
4. The polypeptide of claim 1, modified to be in detectably labeled form.
5. An isolated and purified antibody capable of specifically binding to a polypeptide of claim 1.
6. The antibody of claim 5, wherein the antibody is capable of modulating 25 the biological activity of the polypeptide to which it specifically binds.
7. A hybridoma cell line which produces an antibody of claim 5.
8. An isolated and purified nucleic acid molecule encoding a platelet VDCC α_1 subunit polypeptide of claim 1.
9. The nucleic acid molecule of claim 8, wherein the encoded platelet 30 VDCC α_1 subunit polypeptide comprises a platelet VDCC α_1 S subunit polypeptide or a platelet VDCC α_1 D subunit polypeptide.

10. The nucleic acid molecule of claim 8, wherein the encoded polypeptide comprises:

- (a) a polypeptide having the amino acid sequence set forth as SEQ ID NO:2 or 4;
- 5 (b) a polypeptide that is a biological equivalent of the polypeptide of SEQ ID NOs:2 or 4; or
- (c) a polypeptide which is immunologically cross-reactive with an antibody that shows specific binding with a polypeptide of any of SEQ ID NO:2 or 4.

10. 11. The nucleic acid molecule of claim 8, comprising:

- (a) a nucleic acid molecule having the nucleotide sequence set forth as any of SEQ ID NOs:1 , 3, 5-8, 28, or 29;
- (b) a nucleic acid molecule that is substantially identical to any of SEQ ID NOs:1 , 3, 5-8, 28, or 29;

15. 12. The isolated nucleic acid molecule of claim 8, comprising a 20 nucleotide sequence that is identical to a contiguous 20 nucleotide sequence of SEQ ID NOs:28 or 29.

13. The nucleic acid molecule of claim 8, further defined as a DNA segment.

20. 14. The nucleic acid molecule of claim 13, further defined as positioned under the control of a promoter.

15. The nucleic acid molecule of claim 14, wherein said DNA segment and promoter are operationally inserted into a recombinant vector.

25. 16. A recombinant host cell comprising the nucleic acid molecule of claim 8.

17. The recombinant host cell of claim 16, wherein the cell further comprises a platelet or a megakaryocyte.

18. A method of producing an antibody that specifically binds a platelet VDCC α_1 subunit polypeptide, the method comprising:

30. (a) transfecting a recombinant host cell with a nucleic acid molecule that encodes a platelet VDCC α_1 subunit polypeptide of claim 1;

(b) culturing the host cell under conditions sufficient for expression of the polypeptide;

- (c) recovering the polypeptide; and
- (d) preparing an antibody to the polypeptide, wherein the antibody specifically binds the polypeptide.

19. The method of claim 18, wherein the polypeptide comprises a 5 polypeptide as set forth as SEQ ID NO:2 or 4.

20. The method of claim 18, wherein the nucleic acid molecule comprises a nucleotide sequence as set forth in any of SEQ ID NOs:1 , 3, 5-8, 28, or 29.

21. A method of detecting a platelet VDCC α_1 subunit polypeptide, the method comprising immunoreacting the polypeptide with an antibody prepared 10 according the method of claim 18 to form an antibody-polypeptide conjugate; and detecting the conjugate.

22. An assay kit for detecting the presence of a platelet VDCC α_1 subunit polypeptide in a biological sample, the kit comprising a first antibody that specifically binds a polypeptide of claim 1.

15 23. The assay kit of claim 22, further comprising a second container containing a second antibody that immunoreacts with the first antibody.

24. The assay kit of claim 23, wherein the first antibody and the second antibody comprise monoclonal antibodies.

25. The assay kit of claim 23, wherein the first antibody is affixed to a solid 20 support.

26. The assay kit of claim 23, wherein the first and second antibodies each comprise an indicator.

27. The assay kit of claim 26, wherein the indicator is a radioactive label or an enzyme.

25 28. An assay kit for detecting the presence, in a biological sample, of an antibody that specifically binds a platelet VDCC α_1 subunit polypeptide, the kit comprising a polypeptide of claim 1 that specifically binds the antibody, wherein the polypeptide is present in an amount sufficient to perform at least one assay.

29. A method of detecting a nucleic acid molecule that encodes a platelet 30 VDCC α_1 subunit polypeptide in a biological sample containing nucleic acid material, the method comprising:

- (a) hybridizing the nucleic acid molecule of claim 8 under stringent hybridization conditions to the nucleic acid material of the biological sample, thereby forming a hybridization duplex; and
- (b) detecting the hybridization duplex, whereby a platelet VDCC α_1 subunit polypeptide is detected.

5 30. A method to determine the presence or absence of a mutation conferring altered VDCC α_1 subunit activity in a platelet, said method comprising the step of analyzing a nucleic acid or protein sample for the presence of a mutation in a nucleic acid molecule encoding the platelet VDCC α_1 subunit polypeptide of claim

10 1.

- 31. The method of claim 30, further comprising:
 - (a) amplifying nucleic acid molecules in said sample using a nucleic acid amplification method and primers that selectively amplify said nucleic acid molecule encoding a platelet VDCC α_1 subunit polypeptide; and
 - (b) identifying whether a mutation is present in said amplified nucleic acid molecule.
- 32. The method of claim 31, further comprising the step of analyzing a protein sample for the presence of a mutation in a platelet VDCC α_1 subunit polypeptide.

20 33. A method for detecting a polymorphism in a nucleic acid molecule that encodes a platelet VDCC α_1 subunit polypeptide, the method comprising:

- (a) amplifying nucleic acid molecules in said sample using a nucleic acid amplification method and primers that selectively amplify said nucleic acid molecule encoding a platelet VDCC α_1 subunit polypeptide; and
- (b) identifying whether a polymorphism is present in said amplified nucleic acid molecule.

25 34. A kit for detecting a polymorphism in a nucleic acid molecule encoding a platelet VDCC α_1 subunit polypeptide, the kit comprising:

- (a) a reagent for detecting a polymorphism in a nucleic acid molecule encoding a platelet VDCC α_1 subunit polypeptide in a biological sample; and
- (b) a container for the reagent.

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35. The kit of claim 34, further comprising a reagent for amplifying a nucleic acid molecule encoding a platelet VDCC α_1 subunit polypeptide.

36. The kit of claim 35, wherein the amplifying reagent comprises a polymerase enzyme suitable for use in a polymerase chain reaction and a pair of 5 oligonucleotides.

37. The kit of claim 35, further comprising a reagent for extracting a nucleic acid sample from a biological sample obtained from a subject.

38. A method of screening candidate substances for an ability to modulate platelet VDCC α_1 subunit biological activity, the method comprising:

10 (a) establishing a test sample comprising a nucleic acid molecule encoding a platelet VDCC α_1 subunit polypeptide;

(b) administering a candidate substance to the test sample; and

(c) measuring the interaction, effect, or combination thereof, of the candidate substance on the test sample to thereby determine the 15 ability of the candidate substance to modulate platelet VDCC α_1 subunit biological activity.

39. The method of claim 38, wherein the candidate substance is a candidate protein, a peptide, an antibody, a nucleic acid, or a chemical compound.

40. The method of claim 39, further comprising the step of purifying and 20 isolating a gene encoding the candidate polypeptide.

41. The method of claim 39, wherein the platelet VDCC α_1 subunit polypeptide is contained within cells in cell culture.

42. A recombinant cell line suitable for use in the method of claim 41.

43. The method of claim 38, further comprising a modulatable 25 transcriptional regulatory sequence of a platelet VDCC α_1 subunit polypeptide-encoding gene and a reporter gene which is capable of producing a detectable signal, wherein a candidate substance as a modulator of platelet VDCC α_1 subunit biological activity is based on the amount of signal produced in relation to a control sample.

30 44. The method of 43, wherein the reporter gene encodes the platelet VDCC α_1 subunit polypeptide of claim 1.

45. A method of modulating platelet VDCC α_1 subunit polypeptide biological activity in a cell, the method comprising administering to the cell an

effective amount of a substance capable of modulating activity of a platelet VDCC α_1 subunit polypeptide in the cell to thereby modulate platelet VDCC α_1 subunit polypeptide biological activity in the cell.

46. The method of claim 45, wherein the cell is a platelet or a 5 megakaryocyte.

47. The method of claim 46, wherein the cell comprises a cell in a vertebrate subject.

48. The method of claim 47, wherein the vertebrate subject is a mammal.

49. The method of claim 45, wherein the step of administering further 10 comprises administering an effective amount of a substance that modulates expression of a platelet VDCC α_1 subunit polypeptide-encoding nucleic acid molecule in the cell.

50. The method of claim 45, wherein the substance that modulates the platelet VDCC α_1 subunit biological activity comprises an anti-platelet VDCC α_1 15 subunit polypeptide antibody, a polypeptide, a peptide, a chemical compound, or a nucleic acid.

51. The method of claim 50, wherein the nucleic acid substance that modulates expression of a platelet VDCC α_1 subunit polypeptide-encoding nucleic acid molecule comprises an antisense oligonucleotide.

52. The method of claim 50, wherein the polypeptide, peptide, or chemical compound substance that modulates expression of the platelet VDCC α_1 subunit polypeptide-encoding nucleic acid molecule comprises a ligand for a modulatable transcriptional regulatory sequence of a platelet VDCC α_1 subunit polypeptide-encoding nucleic acid molecule.

53. A pharmaceutical composition comprising a therapeutically effective amount of a modulator of a biological activity of a platelet VDCC α_1 subunit polypeptide, and combinations thereof, and a pharmaceutically acceptable diluent or vehicle.

54. The pharmaceutical composition of claim 53, wherein the platelet 30 VDCC α_1 subunit polypeptide-biological-activity-modulator preferentially binds a platelet VDCC α_1 subunit polypeptide, or a fragment or derivative thereof.

55. A method for modulating calcium transport in a cell, the method comprising introducing to the cell a construct comprising a nucleic acid sequence

encoding a platelet VDCC α_1 subunit polypeptide operatively linked to a promoter, wherein production of the platelet VDCC α_1 subunit polypeptide in the cell results in modulation of calcium transport.

56. The method of claim 55, wherein the construct further comprises a 5 vector selected from the group consisting of a plasmid vector or a viral vector.

57. The method of claim 55, wherein the construct further comprises a liposome complex.

58. The method of claim 55, wherein the cell is a platelet or a megakaryocyte.

10 59. The method of claim 55, wherein the cell comprises a cell in a vertebrate subject.

60. The method of claim 59, wherein the vertebrate subject is a mammal.

61. A transgenic non-human animal having incorporated into its genome a nucleic acid molecule encoding a human platelet VDCC α_1 subunit polypeptide, 15 wherein the human platelet VDCC α_1 subunit polypeptide is expressed in the transgenic non-human animal.

62. A transgenic non-human animal having modified or deleted from its genome a nucleic acid molecule encoding a platelet VDCC α_1 subunit polypeptide.